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Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [*Sorghum bicolor* (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures

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Abstract

Global climate change, especially, increases in carbon dioxide (CO_2) concentration and the associated increases in temperature will have significant impact on the crop production. Grain-sorghum [*Sorghum bicolor* (L.) Moench] cultivar DeKalb 28E was grown at daytime maximum/nighttime minimum temperature regimes of 32/22, 36/26, 40/30 and 44/34 °C at ambient (350 μmol CO_2 mol^{-1}) and elevated (700 μmol CO_2 mol^{-1}) CO_2 from emergence to maturity in controlled environments to quantify the effects of temperature and CO_2 on the reproductive processes and yield. Growth temperatures of 40/30 and 44/34 °C inhibited panicle emergence. Growth temperatures ≥36/26 °C significantly decreased pollen production, pollen viability, seed-set, seed yield and harvest index when compared to 32/22 °C. Percentage decreases in pollen viability, seed-set, seed yield and harvest index due to elevated temperature were greater at elevated CO_2 when compared with ambient CO_2. Elevated CO_2 increased seed yield (26%) at 32/22 °C, but decreased seed yield (10%) at 36/26 °C. At high temperatures, elevated CO_2 increased vegetative growth but not seed yield, thus, leading to decreased harvest index. We conclude that the adverse effects of elevated temperature on reproductive processes and yield of grain-sorghum were more severe at elevated CO_2 than at ambient CO_2; and the beneficial effects of elevated CO_2 decreased with increasing temperature. The adverse temperature sensitivity of reproductive processes and yield at elevated CO_2 was attributed to higher canopy foliage and seed temperatures.

Keywords: Sorghum; Climate change; Global warming; Carbon dioxide; Yield

1. Introduction

Global climate change will pose a serious challenge to crop production across the world. One of the important components of global climate change is increase in the Earth's near-surface temperatures. This increase in temperature is often associated with increases in concentrations of atmospheric carbon dioxide (CO_2) and other heat-trapping greenhouse gases such as methane, nitrous oxide, ozone and water vapor. The concentration of CO_2 near the ground level has risen from about 275 μmol mol^{-1} in the pre-industrial times to 372 μmol mol^{-1} in 2002 (Keeling and Whorf, 2003), an increase of 35%. At the present rate of greenhouse gas

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emissions, atmospheric CO_2 is predicted to double by end of this century (IPCC, 2001) which could increase the surface temperatures in the range of 1.8–5.8 °C (IPCC, 2001). These changes in the climate would have enormous influence on productivity of important food crops in various regions of the world.

Sorghum [*Sorghum bicolor* (L.) Moench] is an important C_4 crop species with a photosynthetic mechanism that is potentially more efficient in using CO_2 (at current concentrations), solar radiation, water and nitrogen when compared to C_3 crops such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and bean (*Phaseolus vulgaris* L.). The optimum mean temperature range for the seed germination of grain-sorghum is 21–35 °C, whereas for the vegetative growth and development it ranges from 26 to 34 °C and for the reproductive growth it is from 25 to 28 °C (Maiti, 1996). There is little knowledge of the effects of temperature on the reproductive growth of sorghum and almost none on the effects of very high temperature (Peacock and Heinrich, 1984). Downes (1972) exposed two grain-sorghum cultivars Milo 7078 and Caprock to five temperature regimes (day/night, 21/16, 24/19, 27/22, 30/25 and 33/28 °C) and found that the maximum dry matter and seed yields were produced at 27/22 °C. Chowdhury and Wardlaw (1978) reported that at temperatures above 30/25 °C, the rate of seed filling in grain-sorghum cultivar Texas 610 was not affected, but the seed-filling duration was decreased resulting in 50% smaller seed size at 33/28 °C. There is a lack of information on the effects of temperatures >33/28 °C on phenology, growth, reproductive processes, yield and yield components in grain-sorghum. Temperatures close to or >32/22 °C commonly occur during the crop life cycle in the semi-arid regions of the world where grain-sorghum is an important component of cropping systems (for example Hyderabad, India; Gedraf, Sudan; Bamoka, Mali; and Lubbock, Texas, USA; Fig. 1). With the anticipated global warming these regions will be subjected to even higher temperatures. Therefore, measurements on impact of high temperature on reproductive growth and yield processes of grain-sorghum are needed.

Studies in controlled environments have shown that doubling of CO_2 could increase the growth, biomass and yield of C_3 crops by 10–50% and that of C_4 crops by 0–10% (Poorter and Navas, 2003). Previous studies on grain-sorghum in green house growth conditions have shown that there is no effect of elevated CO_2 (700 μmol mol^{-1}) when compared to the ambient CO_2 (380 μmol mol^{-1}) on individual leaf area, leaf weight, stem weight, root weight or total vegetative dry weight when harvested before panicle initiation, i.e. about 23, 35 and 41 days after sowing (DAS) (Ziska and Bunce, 1997, 1999).

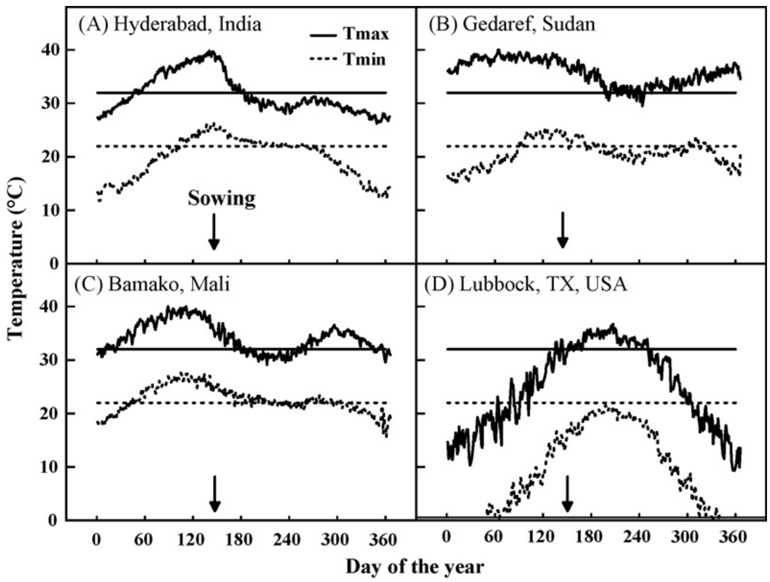

Fig. 1. Daily maximum and minimum temperature during the crop growing season for major sorghum producing regions across the world, such as (A) Hyderabad, India (17°3′N lat., 78°16′E long., 540 m elevation); (B) Gedaref, Sudan (14°3′N lat., 35°04E long., 590 m elevation); (C) Bamako, Mali (12°5′N lat., 7°9′W long., 380 m elevation); and (D) Lubbock, Texas, USA (33°4′N lat., 101°8′W long., 990 m elevation). The data are the daily means for 8–10 years. Weather data were obtained from International Crops Research Institute for the Semi Arid Tropics (ICRISAT, Patancheru, India) and United States National Climate Data Center (Asheville, NC, USA).

However, Free Air Carbon-dioxide Enrichment (FACE) experiments in the field showed that there were small but significant increases in leaf, stem and total biomass at maturity of grain-sorghum at elevated CO_2 (560 μmol mol^{-1}) when compared to the ambient CO_2 (368 μmol mol^{-1}) (Ottman et al., 2001). The effects of elevated CO_2 on grain-sorghum yield under irrigated conditions are variable, some indicating decrease (Ellis et al., 1995), no change (Marc and Gifford, 1984) or increase in yield (Amthor et al., 1994; Reeves et al., 1994). All these studies were conducted at close to optimum temperature; thus, the effects of elevated CO_2 at supra-optimal temperatures on vegetative growth, seed-set, yield and yield components are not well understood.

Although a few studies have investigated the interactive effects of elevated CO_2 and water stress on grain-sorghum (Ottman et al., 2001; Wall et al., 2001), there have been no systematic studies on combined effects of elevated CO_2 and elevated temperature on grain-sorghum. Studies on C_3 crops such as rice (Baker et al., 1995; Matsui et al., 1997), soybean (*Glycine max* (L.) Merril; Baker et al., 1989), dry bean (Prasad et al., 2002), peanut (*Arachis hypogaea* L.; Prasad et al., 2003), cowpea (*Vigna unguiculata* L.; Ahmed et al., 1993), wheat (Wheeler et al., 1996a) and cotton (*Gossypium hirsutum* L.; Reddy et al., 2000) showed no beneficial interactions between elevated temperature and CO_2 on yield. The interaction between elevated temperature and CO_2 on various physiological, growth and yield traits of grain legumes and cotton are reviewed by Prasad et al. (2005) and Reddy et al. (2005), respectively. For most of C_3 crops the higher photosynthetic rates and vegetative growth at elevated CO_2 did not ameliorate the negative effects of high temperature on reproductive processes and yield. However, because of greater temperature tolerance of C_4 crop species for photosynthesis and yield, it is important to test this hypothesis on reproductive processes in a crop such as grain-sorghum.

The objective of this research was to quantify the effects of season-long exposure to a range of high temperatures (daytime maximum/nighttime minimum, 32/22, 36/26, 40/30 and 44/34 °C) at ambient (350 μmol CO_2 mol^{-1}) and elevated (700 μmol CO_2 mol^{-1}) atmospheric CO_2, imposed from emergence to maturity on phenology, leaf photosynthesis, stomatal conductance, pollen production, pollen viability, seed-set, dry matter production, seed yield and yield components of grain-sorghum under fully irrigated conditions. We specifically wanted to test the hypothesis that there are no beneficial effects of elevated CO_2 on sensitivity of reproductive processes, yield and yield components to high temperatures in grain-sorghum.

2. Materials and methods

This research was conducted in the University of Florida and USDA-ARS sunlit controlled environment facilities at the Plant and Soil Science Field Teaching Laboratory of the Agronomy Department at Gainesville, FL, USA.

2.1. Chamber characteristics

Experiments were conducted in eight outdoor soil–plant–atmospheric-research (SPAR) growth chambers, each exposed to the same level of solar radiation. These chambers have unique computer controlled programs, equipment and structure to control air temperature, dew-point temperature and CO_2 at pre-determined set-points. Each chamber is airtight and has an upper aluminum frame measuring 1 m wide, 2 m long and 1.5 m high covered with a polyethylene telephtalate ‘‘six light’’ film (Taiyo Kogyo Co., Tokyo, Japan) walls which enclose the crop canopy. The bottom rooting-chamber (aluminum lysimeter) has the same cross section as the upper frame and is 0.6 m deep. All chambers contain the same natural topsoil of Kendrick sand (loamy, siliceous, Arenic Paleudult) obtained from a nearby field (90.7% sand, 5.6% silt and 3.7% clay). In each chamber, air is circulated from top to the bottom of the canopy chamber using impeller fans located in the external air-circulating ductwork. Each air handling system consists of a chilled water heat exchanger and an electrical resistive heater coil with output regulated by a proportional controller (AA Electric, Lakeland, FL, USA). Chilled water was provided by two chiller systems (York International, York, PA, USA).

Dry bulb air temperatures were measured with aspirated and shielded copper–constantan thermocouples and controlled to set-points by electrical resistance proportional heaters located in the external air circulation ductwork. Air dewpoint temperatures were measured using relative humidity and temperature transmitter sensors (Model HMD70Y, Vaisala, Woburn, MA, USA) and controlled to set-points with bypass valves that determine the flow of chilled-water through heat exchangers. Air temperature and dewpoint temperature were typically controlled according to a sinusoidal wave function during the day and a logarithmically decreasing (decay) function during the night (Parton and Logan, 1981). The dewpoint temperatures were maintained 10 °C below target mean dry bulb air temperature. These environments provided nearly constant relative humidities (55–58%) at 15:00 h in all treatments. Constant relative humidities were chosen, as relative humidities were predicted to stay constant under climate change conditions (Rind, 1998).

Daytime CO_2 was measured continuously on samples from each chamber using infrared gas analyzers (Model Ultramat 21P, Siemens, New York, NY, USA) and controlled at a given set points by injecting pure CO_2 from a high pressure cylinder through a mass flow controller (Model 5850i, Brooks Instruments, Hatfield, PA, USA). CO_2 was not controlled during nighttime, but vents were opened in the external air-circulating duct-work for 13 min every hour to remove excess CO_2 produced from respiration. Incoming solar radiation was measured using a pyranometer and calibrated solar cells (Panasonic, Atlanta, GA, USA) on each growth chamber. Canopy foliage temperature was measured using infrared sensors (Model OS550 Series, Omega, Stanford, CT, USA).

Each chamber was controlled by a Campbell CR10X processor (Campbell Scientific Inc., Logan, Utah). Control instructions were programmed at central host-processor computer using Campbell Loggernet software, and communicated to each CR10X via coaxial cable. Measurements of all parameters were taken every 10 s, means were stored at 5-min intervals and all data were downloaded automatically to a host processor via coaxial cables every day (24 h). Details of the chamber characteristics and functions, methods and quality of specific chamber environment and controls are described in several publications (Pickering et al., 1994; Prasad et al., 2003; Allen et al., 2003).

In addition the chamber system was equipped with a separate computer controlled datalogger (Gaffney Instruments, Gainesville, FL, USA) to measure seed or rachis temperatures with micro-thermocouples (Model MT-29/1B, Physiotemp Instruments Inc., Clifton, NJ, USA). Thermocouples were inserted into the seed, except in treatments with no seeds where the thermocouples were inserted into main rachis. Temperatures were measured and stored at 15 min intervals throughout the seed-growth period.

2.2. *Quality of environmental control and chamber performance*

Before start of the main experiment, several short-term experiments were conducted over a period of 15 days to test the uniformity and quality of environmental conditions (dry bulb temperatures and CO_2 concentrations) across all growth chambers. This was done by exposing all eight chambers to a single set-point temperature of 32/22, 36/26, 40/30 or 44/34 °C for 3 successive days. The results of these experiments clearly showed that there was precise control and high uniformity of environmental conditions across all the growth chambers (Fig. 2). When controlled at similar temperature regimes, all chambers had the air temperatures within ±0.1 °C of the target set point temperatures (Fig. 2A). Similarly, all chambers controlled the CO_2 concentration within ±5 μmol mol^{-1} of the target set-point at both ambient (350 μmol mol^{-1}) and elevated levels (700 μmol mol^{-1}) (Fig. 2B). Due to the proximity of all the eight chambers (within 15 m from each other), they received similar photosynthetic photon flux density

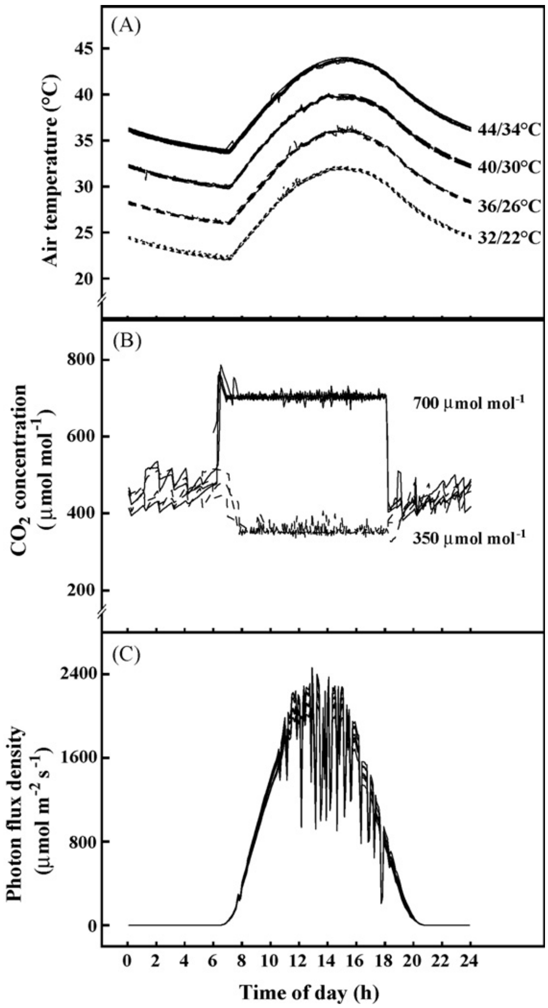

Fig. 2. Target and measured (A) air temperatures in eight different soil–plant–atmosphere-research chambers at set points of 32/22, 36/26, 40/30 and 44/34 °C; and (B) CO_2 concentrations when all chambers were controlled at ambient (350 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) levels at temperature regime of 32/22 °C; and (C) measured photosynthetic photon flux density (PPFD) in eight different chambers at 32/22 °C and ambient CO_2 concentration during a 24 h period. Each datum or line is represented by eight overlapping points from eight different chambers.

(PPFD) (Fig. 2C) through natural sunlight. The PPFD transmission through each chamber was between 85 and 88%. During most of the crop season PPFD was >1600 μmol m^{-2} s^{-1} during the day. The soil in all chambers was from the same field, and had a good capillarity flow. The soil preparation, fertilizer, irrigation schedule and other crop management practices were similar in all chambers. Thus, there were no differences among the chambers in environmental control and crop management conditions.

To test the performance of growth chambers, two experiments were conducted under similar environmental and crop management conditions. The performance of the chamber was tested from the measured data on growth and dry matter production of crops in each chamber. In these experiments, all the eight chambers were controlled at similar environments [daytime maximum/nighttime minimum dry bulb temperature regimes of 32/22 °C (Exp 1) or 30/20 °C (Exp 2), at ambient CO_2 of 350 μmol mol^{-1}] from sowing to the final harvest under similar crop management practices as described below. Data on various growth and yield traits were measured on five randomly selected plants at various stages of development. The methods used for data collection were similar to that of current experiment and are described in subsequent sections. The data analysis was conducted using analysis of variance techniques in Statistical Analysis System (SAS, 2003) software. The statistical analyses of the data showed that there were no significant differences between the eight chambers for all growth and yield traits, such as individual plant height ($P = 0.64$), leaf numbers ($P = 0.06$), total leaf area ($P = 0.36$), leaf dry weight ($P = 0.47$), stem dry weight ($P = 0.35$), total dry weight ($P = 0.74$), percent pollen viability ($P = 0.22$) and percent seed-set ($P = 0.96$) of grain-sorghum when grown directly in soil bins and harvested at 47 DAS (Exp 1). The time to panicle emergence was also similar (35–36 DAS) in all eight chambers. In the second experiment where grain-sorghum and corn (*Zea mays* L.) were grown in 15 L pots that were placed inside each growth chamber and harvested at 21 DAS (Exp 2) also showed similar results. There was no significant differences (all $P > 0.15$) between the growth chambers for plant height, leaf numbers, total leaf area and dry matter production of either grain-sorghum or corn. Overall, the data from these two experiments clearly demonstrates that all chambers performed similarly and produce similar results in terms of plant development, growth and dry matter production when grown under similar environmental and crop management conditions. In addition the quality of the environmental control in all chambers was similar (Figs. 2 and 3). Together, these data provide improved assurance on uniformity of the chambers, chamber-conditions and reliability of data collected from experiments in SPAR growth chamber without true replications. This is particularly important because often it is not possible to have multiple replications for experiments conducted in growth chambers due to limitation of cost, time and number of treatments.

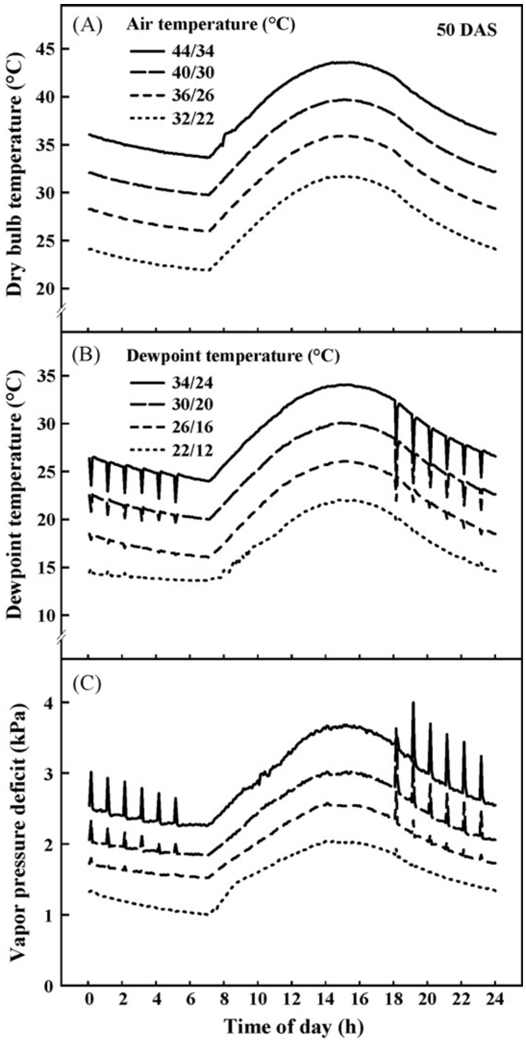

Fig. 3. Measured chamber (A) air (dry bulb) temperatures; (B) dewpoint temperatures; and (C) difference between saturated and actual vapor pressure (air vapor pressure deficit, VPD) during 24 h period at 50 days after sowing in 2003, for chambers with set point air temperatures of 32/22, 36/26, 40/30 and 44/34 °C at elevated CO_2 concentration (700 μmol mol^{-1}).

2.3. Plant husbandry

Uniform seeds of the semi-dwarf and photoperiod-insensitive sorghum cultivar DeKalb 28E were treated with fungicide Captan [*cis-N*-(trichloromethyl) thio-4-cyclohexane-1,2-di-carboximide] as a precautionary measure against seed-borne diseases. Four seeds per hill were sown by hand on 11 August 2003 at a depth of 2 cm. Two rows, each 20 cm apart were sown running along 2-m length (East-West) and plants within the row were spaced 9 cm apart. Plants were irrigated by overhead sprinkler irrigation from sowing to 10 days after emergence until a good root system was established. Thereafter, plants were fully dependent on subsurface irrigation provided by a constant water table at 45 cm beneath the soil surface maintained by an external float-valve device inside a bucket for each chamber. There was a good capillary flow through the soil, which kept the soil near field capacity throughout the growing season. Soon after emergence, plants were thinned and a uniform population of 20 plants per square meter (40 per chamber) was maintained. The crop was fertilized with 120 g N, 20 g P and 20 g K per m^2 provided through inorganic fertilizers. All P and K and 50% of N (60 g per chamber) was given as basal dose at the time of sowing and the remaining 50% of N was provided in two split applications of 30 g m^{-2} each at panicle initiation and flowering. Weeding was done by hand as necessary. There was no evidence of pests, diseases or nutrient stress during the crop-growing season. Carbaryl (1-naphthalenylmethylcarbamate) dust was applied as a precautionary measure to prevent potential insect damage.

2.4. Temperature and CO_2 treatments

Carbon dioxide was controlled and maintained at 350 μmol mol^{-1} in all the chambers from sowing to appearance of the first leaf (5 DAS). Thereafter, CO_2 concentration in four chambers was increased to 700 μmol mol^{-1}. From sowing to full emergence (7 DAS), the temperature was set at 36/26 °C in all chambers. Thereafter, four temperature treatments of 32/22, 36/26, 40/30 and 44/34 °C were randomly allocated to the eight chambers in paired sets (one each at 350 and 700 μmol mol^{-1}). The CO_2 and the temperature control continued until final harvest at seed maturity.

2.5. Gas exchange measurements

Individual attached leaf gas exchange measurements, i.e. photosynthesis, stomatal conductance and transpiration were measured at the time of panicle emergence using LI-COR 6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA). Gas exchange measurements were made during midday at respective growth temperature and CO_2 conditions. The growth chamber conditions were simulated in the leaf chamber of photosynthesis system through integrated peltier temperature controller and CO_2 injection system. Measurements were conducted on three topmost fully expanded leaves from three different plants on a clear sunny day. The internal light source (6400-02B red/blue) in the LI-COR 6400 was set at 1800 μmol m^{-2} s^{-1} to have constant and uniform light across all measurements.

2.6. Pollen production, pollen viability and seed-set

At the time of panicle emergence, individual panicles were tagged to estimate pollen production, pollen viability and seed-set at anthesis. The numbers of pollen grains per anther were counted. Five anthers from five different plants were collected before anthesis (06:30–07:00 h) and all the pollen grains from each anther were squeezed into a separate clean slide with a 10 μL of distilled water. Then the pollen grains were thoroughly dispersed on the slide with a micropipette. The slide was carefully covered with glass slip and the total numbers of pollen grains were counted using a light microscope.

Pollen viability was estimated by two techniques (a) vital stain technique; and (b) *in vitro* pollen germination in artificial pollen germinating medium of Tunistra and Wedel (2000) with slight modifications as described below. At the time of anthesis 5–10 florets were collected at sunrise (between 07:30 and 08:00 h) from five plants. Pollen grains were squeezed from the anthers by tweezers and pollen was collected on clean slides. Pollen viability was tested using 2% tri-phenyl tetrazolium chloride stain. A drop of tetrazolium chloride was added to the dispersed pollen. Tetrazolium chloride stains the live pollen with reddish purple color due to the formation of insoluble red formazan. The numbers of pollen grains stained was recorded 30 min after staining. The percentage of viable pollen was estimated as the percentage of total pollen that was stained.

For *in vitro* pollen germination studies the medium consisted of 150 mg H_3BO_3, 500 mg $Ca(NO_3)_2{\cdot}4H_2O$, 200 mg $MgSO_4{\cdot}7H_2O$, 100 mg KNO_3 and 300 g sucrose dissolved in 1 L of deionized water to which 15 g of agar L^{-1} was added and slowly heated on a hotplate until the agar was completely dissolved. The germinating medium was poured into petri dishes and incubated at 28 °C for 30 min. The pollen grains were collected from five plants and evenly distributed on five petri dishes smeared with the pollen-germinating medium and kept in the dark for 45 min. The percentage of pollen germination was estimated by counting the total number of pollen grains and number of germinated pollen in three random microscopic fields in each petri dish. The pollen was considered germinated if the length of the pollen tube was greater than the diameter of the pollen grain.

At maturity, the numbers of filled and unfilled seeds were estimated on the tagged panicles. Individual florets were checked for seed by pressing the floret between the thumb and the index finger. Seed-set was estimated as the ratio of florets with seed to the total number of florets, and expressed as a percentage.

2.7. Seed growth rates and seed filling duration

To estimate individual seed growth rates, primary branches were tagged at the time of flowering, and thereafter the tagged florets were harvested every 7 days and the dry weight of seeds measured after oven-drying the samples. At each date, five seeds from five different plants were harvested. Rate of seed filling was estimated by regression of seed weight over time. Based on regression lines, duration of seed filling was estimated as the time from start of seed growth to the time when the seeds reached maximum size.

2.8. Dry matter production, yield and components of yield

At maturity eight plants (four plants from each row) were randomly selected and the data on component part dry weights (leaf, stem, root and head) were recorded. Leaf, stem and root were dried at 60 °C for 7 days. Panicle length and diameter were measured. Panicles were separated and the total numbers of branches, total number of filled and unfilled grains (seeds) were counted to obtain data on percentage seed-set. Dry weights of seed components were recorded after drying at 35–40 °C for 10 days to allow tests on subsequent seed germination and seedling vigor. Harvest index was estimated as the ratio of seed yield to total dry matter yield. Data on weight per seed were determined based on seed numbers and dry weights.

All the remaining plants were harvested, panicles separated and data on seed weight and total vegetative dry weight (leaf, stem and root) were recorded after oven drying for 7 days. Results of statistical analysis of data on growth and yield from the whole plants m^{-2} land area and eight single-plant samples were similar. Mean data on reproductive processes such as percentage seed-set, panicle morphology, seed growth and components of yield are available only from eight plant samples. Therefore, data on all other traits are also presented based on results from these eight single plant samples.

2.9. Data analyses

All data were statistically analyzed by PROC GLM procedures in Statistical Analysis System (SAS, 2003) software. There were eight replications (eight different plants) for seed-set, growth and yield data. There were five replications for pollen analysis (production and germination). The following statistical model, $y = \beta_0 + \beta_1 T + \alpha_1 CO_2 + \alpha_2 T \times CO_2$, was used to test the significance of the effects of temperature, and CO_2 and their interaction. Temperature and CO_2 were used as classified variables. Standard error of means of eight plant samples for each datum is shown as an estimate of variability. Comparison of regressions was used to test if the slopes and the intercepts were significantly different.

3. Results

The average seasonal diurnal day/night temperatures were within ±0.14 °C of the target temperatures under both ambient and elevated CO_2 chambers. The average seasonal daytime CO_2 in ambient and elevated CO_2 chambers were 358 ± 0.9 and 696 ± 1.2 μmol mol^{-1}, respectively, when averaged across the respective chambers. The measured dewpoint temperatures across all treatments were within 0.2 °C of the target for all chambers. The measured diurnal dry bulb temperatures, dewpoint temperatures and vapor pressure deficits (VPD) for all four air (dry bulb) temperature regimes at 50 DAS are shown in Fig. 3. There was no difference between measured dewpoint and VPD data between ambient and elevated CO_2, therefore, only the data from elevated CO_2 are shown. At 15:00 h when the daytime maximum dry bulb temperatures of 32, 36, 40 and 44 °C and dewpoint temperatures of 22, 26, 30 and 34 °C occurred, the vapor pressure deficits were 2.1, 2.5, 3.1 and 3.8 kPa, respectively (Fig. 3). Similarly, at 07:00 h when the daytime minimum dry bulb temperatures of 22, 26, 30 and 34 °C and minimum dewpoint temperatures of 12, 16, 20 and 24 °C occurred, the vapor pressure deficits were 1.2, 1.6, 1.9 and 2.3 kPa, respectively. The hourly spikes in dewpoint temperatures and VPD during the nighttime were due to hourly venting of chambers to remove excess CO_2 from nighttime respiration. Similar study on soybean with same temperature regimes indicated that the vapor pressure deficits linearly increased from 08:00 to 15:00 h during the day across all temperatures (Allen et al., 2003).

There were significant effects of temperature, CO_2 and interaction between temperature and CO_2 on various traits measured in this study. The levels of significance and P values of these effects are shown in Table 1. The specific responses are presented in figures and statistically compared.

3.1. Leaf photosynthesis, stomatal conductance and transpiration

Increases in temperature from 32/22 to 44/34 °C decreased ($P < 0.01$) leaf photosynthesis by 4% when averaged across CO_2 treatments (Fig. 4A). Elevated CO_2 increased ($P < 0.001$) leaf photosynthetic rates across all temperatures by about 9%. There was a linear increase ($P < 0.01$) in stomatal conductance (Fig. 4B) and transpiration rates (Fig. 4C) with increase in temperature from 32/22 to 44/34 °C. Elevated CO_2 decreased ($P < 0.01$) stomatal conductance by about 43% and transpiration by 34% when averaged across all temperatures. The percentage decrease in transpiration due to elevated CO_2 was greater at the highest temperature (44/34 °C). There were no interactions between temperature and CO_2 on leaf photosynthesis or stomatal conductance.

3.2. Phenology

Panicle emergence did not occur at the highest temperature (44/34 °C) until the final harvest at 110 DAS. At 40/30 °C partial panicle emergence occurred in 10% of the plants at 60 days after sowing at ambient or elevated CO_2. At 32/22 and 36/26 °C, panicle emergence occurred at 41 and 40 DAS, respectively, under ambient CO_2. Elevated CO_2 delayed

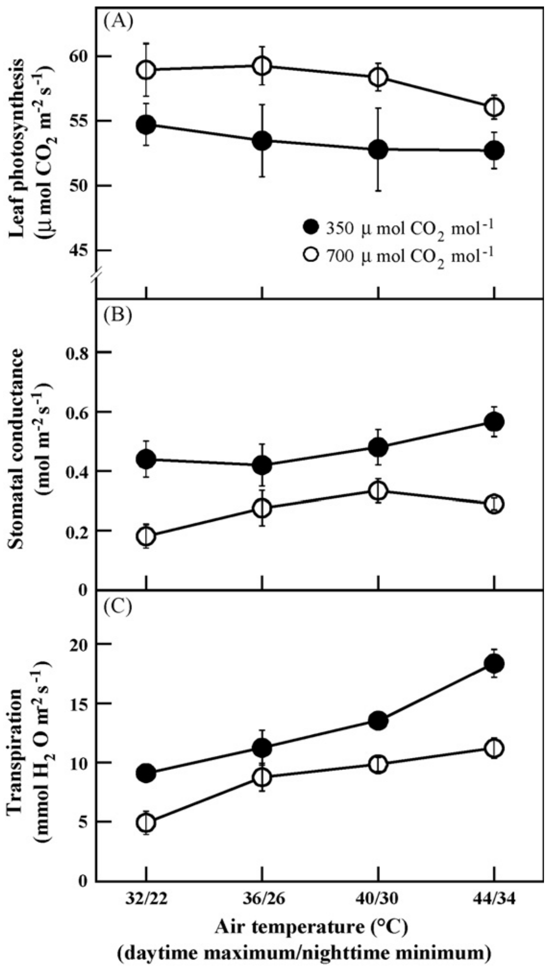

Fig. 4. Influence of growth temperature and CO_2 on attached leaf (A) photosynthesis; (B) stomatal conductance; and (C) transpiration of grain-sorghum. Error bars indicate standard errors.

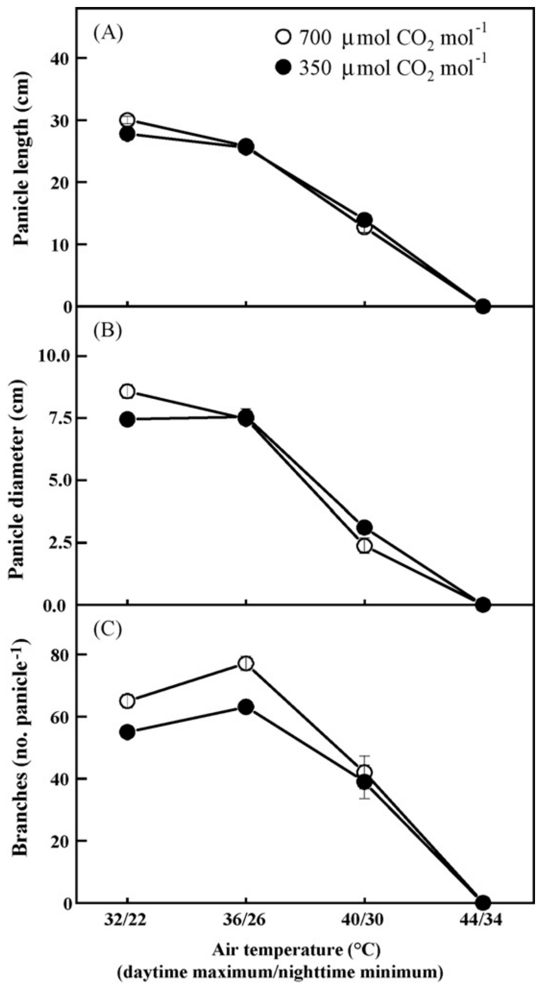

Fig. 5. Influence of growth temperature and CO_2 on (A) panicle length; (B) panicle diameter; and (C) number of branches per panicle of grain-sorghum. Panicles were not produced at 44/34 °C either at ambient or elevated CO_2. Error bars indicate standard errors.

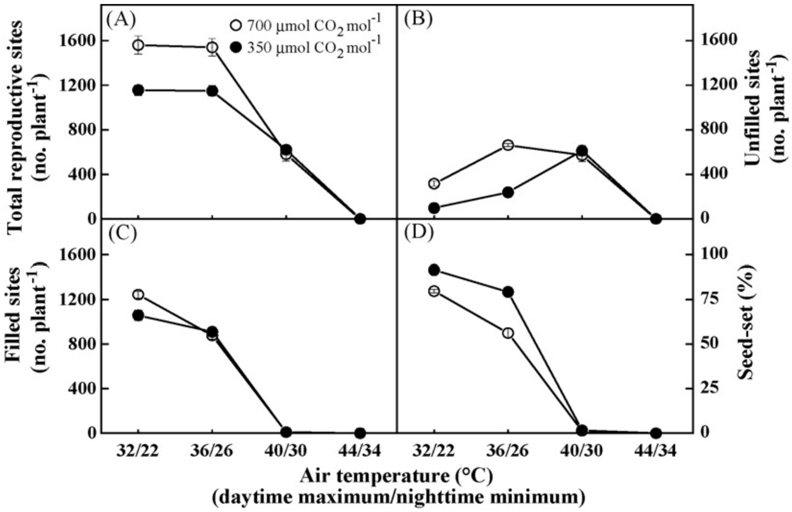

Fig. 6. Influence of growth temperature and CO_2 on (A) total number of reproductive sites; (B) number of unfilled sites; (C) number of filled sites; and (D) percent seed-set of grain-sorghum. Error bars indicate standard errors.

panicle emergence by 4 days at 36/26 °C, but no delay occurred at 32/22 °C. Anthesis occurred 3–4 days after panicle emergence at both temperatures and CO_2 treatments.

3.3. Reproductive morphology and seed-set

Increases in temperature from 32/22 to 36/26 °C decreased ($P < 0.001$) panicle length and panicle diameter by 11%, thereafter panicle length (Fig. 5A) and panicle diameter (Fig. 5B) were decreased linearly by 3.2 and 0.9 cm, respectively, for every °C rise in temperature until 44/34 °C. Panicle length was not influenced by elevated CO_2 or the interaction between temperature and elevated CO_2.

As temperature increased from 32/22 to 36/26 °C, number of branches per panicle was increased ($P < 0.001$) by about 17% at ambient and elevated CO_2, but a further increase in temperature to 40/30 °C decreased the number of branches per panicle (Fig. 5C). Elevated CO_2 increased the number of branches by 18 and 22%, at 32/22 and 36/26 °C respectively, but not at 40/30 °C.

The numbers of unfilled sites increased ($P < 0.001$) with temperature until 40/30 °C at which point all sites were unfilled and there were no sites formed at 44/34 °C (Fig. 6B). Elevated CO_2 increased ($P < 0.001$) the total number of reproductive sites by about 35% at 32/22 and 36/26 °C, but there was no effect at 40/30 °C (Fig. 6A).

Increases in temperature from 32/22 to 36/26 °C decreased number of filled sites (Fig. 6C) and percent seed-set (Fig. 6D) by 14 and 30%, respectively, at

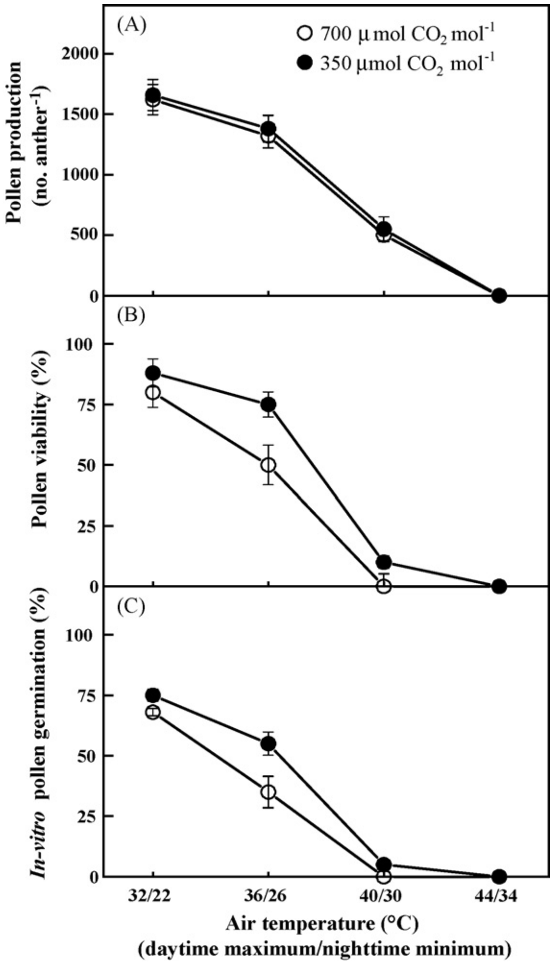

Fig. 7. Influence of growth temperature and CO_2 on (A) pollen production; (B) pollen viability; and (C) *in vitro* pollen germination of grain-sorghum. Error bars indicate standard errors.

Table 1
Significance of the effects of temperature, CO_2 and interaction between temperature and CO_2 on traits and processes of grain-sorghum cultivar DeKalb 28E

Trait (units)	Temperature	CO_2	Interaction
Leaf photosynthesis (μmol m^{-2} s^{-1})	0.007**	<0.001***	0.543
Stomatal conductance (mol m^{-2} s^{-1})	0.006**	<0.001***	0.474
Transpiration (mmol m^{-2} s^{-1})	0.006**	<0.001***	0.03*
Panicle length (cm)	<0.001***	0.492	0.086
Panicle diameter (cm)	<0.001***	0.569	0.001**
Panicle branches (no. $panicle^{-1}$)	<0.001***	0.671	<0.001***
Total reproductive sites (no. $plant^{-1}$)	<0.001***	<0.001***	<0.001***
Unfilled sites (no. $plant^{-1}$)	<0.001***	<0.001***	<0.001***
Filled sites (no. $plant^{-1}$)	<0.001***	0.221	0.07
Seed-set (%)	<0.001***	<0.001***	<0.001***
Pollen production (no. $anther^{-1}$)	<0.001***	0.252	0.526
Pollen viability (%)	<0.001***	0.02*	0.03*
In vitro pollen germination (%)	<0.001***	0.03*	0.005**
Total vegetative dry weight (g $plant^{-1}$)	<0.001***	0.010**	0.041*
Seed dry weight (g $plant^{-1}$)	<0.001***	0.334	0.002**
Harvest index	<0.001***	0.002**	0.002**
Seed-size (mg $seed^{-1}$)	<0.001***	0.779	0.749

(*), (**), (***), Significant at $P < 0.05$, <0.01 and <0.001, respectively.

ambient and elevated CO_2. Elevated CO_2 increased the number of filled sites at 32/22 °C but had no effect at 36/26 °C. There was a negative interaction between temperature and CO_2 on pollen viability. Elevated CO_2 decreased seed-set at 32/22 and 36/26 °C by 13 and 29%, respectively. At 40/30 °C there was no seed-set at either ambient or elevated CO_2.

3.4. Pollen production and pollen viability

There was no effect of CO_2 and no interaction between temperature and CO_2 on pollen production. Increase in temperature decreased ($P < 0.001$) pollen production irrespective of CO_2 (Fig. 7A). Pollen viability as measured by staining technique (Fig. 7B)

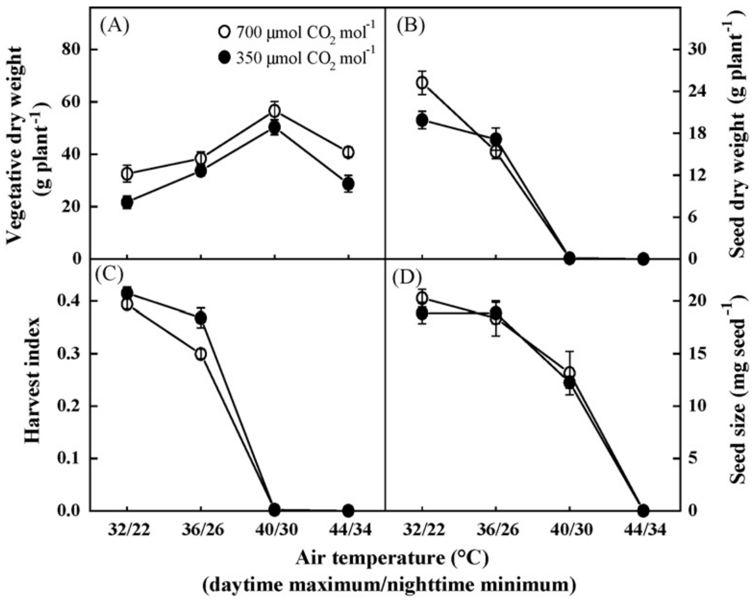

Fig. 8. Influence of growth temperature and CO_2 on (A) total vegetative dry weight (leaf, stem and root); (B) seed yield; (C) harvest index; and (D) seed-size (single seed weight) of grain-sorghum. Error bars indicate standard errors. At 40/30 °C temperature, only two to three plants of the total eight samples had seed-set, therefore, only average of those two to three plants are shown in this figure.

and *in vitro* pollen germination (Fig. 7C) was influenced by temperature ($P < 0.001$), CO_2 ($P < 0.05$) and the interaction between temperature and CO_2 ($P < 0.05$). There was a negative interaction between temperature and CO_2 on pollen viability. Increase in temperature from 32/22 to 36/26 °C decreased pollen germination by 26% at ambient CO_2 and by 48% at elevated CO_2 (Fig. 7C). Elevated CO_2 decreased pollen germination by 9% at 32/22 °C and by 36% at 36/26 °C.

3.5. Dry matter production, seed yield and harvest index

Growth at 40/30 °C significantly increased vegetative dry weight by 120 and 60% at ambient and elevated CO_2, respectively, when compared to other temperatures (Fig. 8A). Elevated CO_2 increased ($P < 0.01$) total dry weights by about 34% when averaged across all temperatures, the beneficial effects were greater at 32/22 and 44/34 °C when compared to other temperature regimes.

There were effects of temperature ($P < 0.001$) and interaction between temperature and CO_2 ($P < 0.01$) but not CO_2 on seed yields (Fig. 8B). There was a negative interaction between temperature and CO_2 on seed yield. At ambient CO_2, increase in temperature from 32/22 to 36/26 and 40/30 °C decreased seed yield by 10 and 99.4%, respectively. The corresponding decreases at elevated CO_2 were 40 and 99.6%, respectively. Elevated CO_2 increased seed yield by 26% at 32/22, but decreased seed yield by 10% at 36/26 °C.

Harvest index was influenced by temperature ($P < 0.001$), CO_2 ($P < 0.01$) and interaction between temperature and CO_2 ($P < 0.01$) (Fig. 8C). There was a negative interaction between temperature and CO_2 on harvest index. As temperature increased from 32/22 to 36/26 °C, harvest index was decreased by 11% at ambient CO_2 and by 24% at elevated CO_2. Elevated CO_2 decreased harvest index by 5 and 19% at 32/22 and 36/26 °C, respectively.

3.6. Seed size, seed growth rate and seed filling duration

There was no effect of elevated CO_2 or interaction between temperature and CO_2 on seed size. Increase in temperature from 32/22 to 44/34 °C significantly ($P < 0.001$) decreased the seed size (Fig. 8D).

Individual seed growth rate and seed filling duration were influenced by temperature ($P < 0.001$) but not by CO_2 or by the interaction between temperature and CO_2. Increase in temperature from 32/22 to 36/26 °C increased seed growth rate from 0.65 to 0.69 mg day^{-1}, but a further increase in temperature to 40/30 °C deceased seed growth to 0.59 mg day^{-1} at ambient or elevated CO_2 (Fig. 9). In contrast, the seed filling duration at 32/22 °C was 34 days and an increase in temperature to 36/26 °C decreased the seed filling duration by 2 days, and a further increase to 40/30 °C decreased it by an additional 6 days. Therefore, the seed size was governed primarily by the seed filling duration, and secondarily by the seed growth rate.

3.7. Canopy foliage and seed temperatures

Elevated CO_2 increased the mean daytime (07:00–19:00 h) foliage temperatures by 1.3, 2.6, 2.7 and 1.2 °C

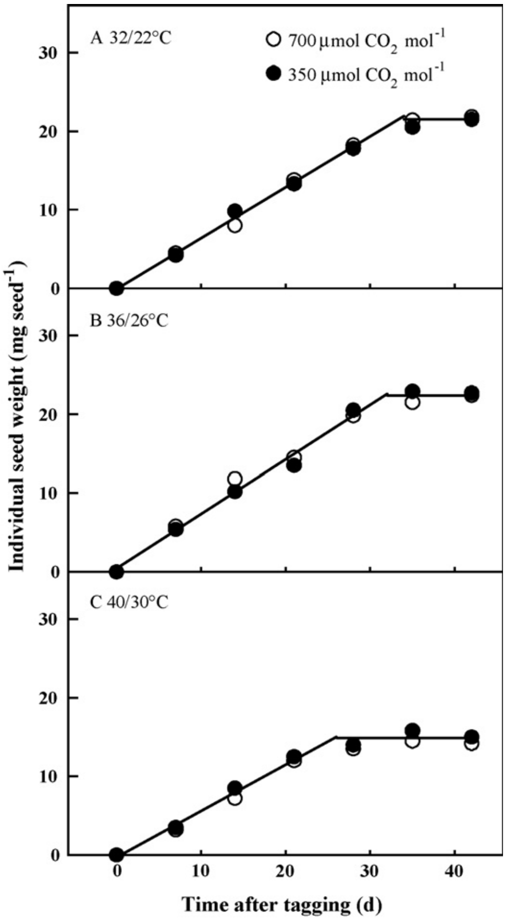

Fig. 9. Time series data on seed weight of grain-sorghum grown at ambient (350 solid symbols) or elevated (700 closed symbols) at daytime maximum and nighttime minimum temperature regimes of (A) 32/22 °C; (B) 36/26 °C; and (C) 40/30 °C. At 40/30 °C, only a few plants (<10%) had panicle emergence and had very low seed-set (2%), which were used for single seed growth data. Regression for sloping lines (a) $y = 0.65x - 0.08$, $r^2 = 0.99$, $n = 10$; (b) $y = 0.69x + 0.44$, $r^2 = 0.99$, $n = 10$; and (c) $y = 0.59x - 0.33$, $r^2 = 0.99$, $n = 8$. Error bars indicate standard errors.

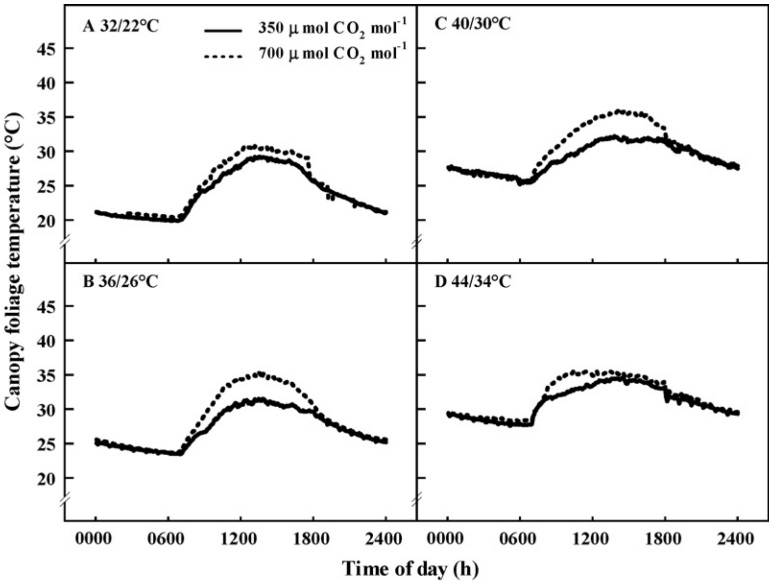

Fig. 10. Measured canopy foliage temperature (°C) during 24 h period under ambient and elevated CO_2 at different temperatures treatments (A) 32/22 °C; (B) 36/26 °C; (C) 40/30 °C; and (D) 44/34 °C. Data are the mean of 60 days period (35–95 DAS) when canopy was fully developed and covered the chamber.

at air temperature regimes of 32/22, 36/26, 40/30 and 44/34 °C, respectively, when averaged for a period of 60 days after canopy closure (Fig. 10). Similarly, mean daytime seed temperatures (measured by micro-thermocouples) at elevated CO_2 were about 1.2, 1.4 and 0.6 °C greater at 32/22, 36/26 and 44/34 °C, respectively, over a 30 days period during the seed growth (Fig. 11).

4. Discussion

The major findings of this study on grain-sorghum are (a) direct temperature effects are important and there were negative interactions between temperature and CO_2 on pollen viability, seed-set, seed yield and harvest index. Adverse effects of elevated temperature on these traits were more severe at elevated CO_2 than at ambient CO_2; (b) the beneficial effects of elevated CO_2 on yield decreased with increasing temperatures; and (c) temperatures of 40/30 and 44/34 °C inhibited panicle emergence and elevated CO_2 delayed panicle emergence at 36/26 °C.

Elevated CO_2 decreased seed-set and pollen viability of sorghum plants when compared to ambient CO_2 (Figs. 6 and 7). There were negative interactions between elevated temperature and CO_2 on the reproductive processes and on the yield of grain-sorghum. Perhaps because elevated CO_2 increased numbers of total reproductive sites, it caused a significant reduction in percent seed-set. The lower seed-set at elevated CO_2 was also reflected in decreased seed yields and harvest index (Fig. 8). These results suggest that the effect of elevated CO_2 on yield is dependent upon temperature. At cooler temperatures (32/22 °C) the effect of CO_2 is positive and resulted in higher yields (26% increase), while at supra-optimal temperatures (36/26 °C) the seed yields were lower (10% decrease), when compared to ambient CO_2. The greater sensitivity of pollen viability and seed-set to increased temperature at elevated CO_2 was indirectly driven by increases in tissue temperature (Figs. 10 and 11) caused by partial closure of stomata and increased leaf resistance to water vapor loss. However, when the data on reproductive processes is quantified in terms of tissue temperatures, it shows no direct influence of elevated CO_2 on these processes. This suggests that elevated CO_2 per se does not influence the reproductive processes, but is the result of associated increase in tissue temperatures under elevated CO_2.

This study suggests that 32/26 °C is near the upper limit for satisfactory reproductive processes for this cultivar of sorghum. Our previous study on dry bean also indicated that the ceiling temperature (where seed-set was zero) for seed-set at elevated CO_2 was 2 °C lower than the value at ambient CO_2 (Prasad et al., 2002). Foliage temperature of canopies grown at elevated CO_2 was reported to be 1–2 °C greater than those at ambient CO_2 for dry bean (Prasad et al., 2002) and soybean (Allen et al., 2003). Greater yield reductions at elevated CO_2 at elevated temperatures were also observed on rice (Kim et al., 1996; Matsui et al., 1997).

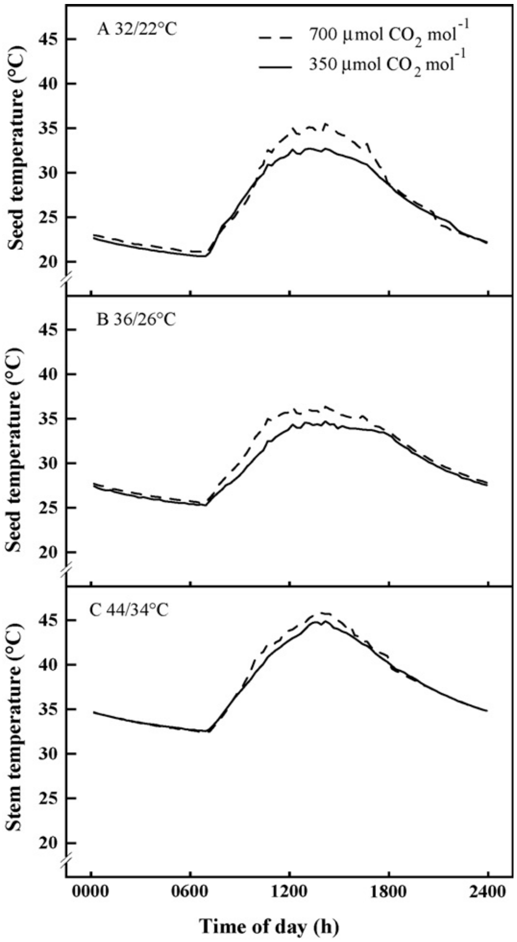

Fig. 11. Measured seed temperature (°C) during 24 h period under ambient and elevated CO_2 at different temperature treatments (A) 32/22 °C; and (B) 36/26 °C. Data are the mean of 30 days (60–90 DAS) during seed growth. There was no panicle emergence at 44/34 °C, however, the culm (stem) temperature was measured for that treatment (C). Data for 40/30 °C were lost due to equipment failure at 80 days after sowing.

The lower seed-set at high temperatures in this study was due to lower pollen production and lower pollen germination, however, the role of stigma receptivity can not be completely overruled. Studies on dry bean (Prasad et al., 2002) and peanut (Prasad et al., 1999, 2000, 2001, 2003), wheat (Saini and Aspinall, 1982) and tomato (*Lycopersicon esculentum* Mill; Sato et al., 2000) showed impairment of pollen at high temperatures. Our recent studies on rice showed that high temperatures decreased both pollen production and pollen reception by stigma leading to lower spikelet fertility and fewer filled grains (Prasad et al., 2006). Furthermore, there were cultivar differences within various rice species and/or ecotypes (Prasad et al., 2006). Decreased pollen production at high temperatures may be related to anther indehiscence (Porch and Jahn, 2001). Lower pollen viability at high temperatures could be related to degeneration of tapetum layer (Suzuki et al., 2001), and/or decreased carbohydrate metabolism (Datta et al., 2001; Pressman et al., 2002; Karni and Aloni, 2002), all of which could significantly influence nourishment of pollen mother cells which could lead to infertile pollen. Identifying the processes that control pollen production and pollen fertility at high temperature would be essential to improve current cultivars and/or to develop new heat tolerant grain-sorghum cultivars.

Elevated CO_2 delayed panicle emergence, flowering and start of seed-filling at high temperatures. Similarly, growth temperature of 40/30 °C delayed panicle emergence. Craufurd et al. (1998) reported that the optimum temperature for panicle initiation ranged from 26 to 27 °C and that supra-optimum temperature delayed panicle initiation in nine different genotypes of grain-sorghum. It appears that the greater delay in panicle initiation and flowering caused by high CO_2 could be attributed to warmer foliage temperature. Stomatal sensitivity to elevated CO_2 may differ among cultivars. It is possible that the cultivars with less stomatal sensitivity to elevated CO_2 may have smaller increases in leaf temperatures, thus, avoiding greater negative impacts on reproductive processes and yield. However, this hypothesis needs to be tested and studies should focus on identifying cultivars of grain-sorghum with less stomatal sensitivity to elevated CO_2.

There was no difference in the rate of seed-filling at 32/22 and 36/26 °C, but the duration of seed filling was slightly decreased by 2 days at 36/26 °C (Fig. 9). At 40/30 °C both the rate of seed-filling and the duration of seed filling were decreased when compared to cooler temperatures. Chowdhury and Wardlaw (1978) reported that increasing temperatures from 24/19 to 35/28 °C slightly increased the rate of seed-filling but drastically reduced the duration of seed-filling from 42 to 18 days. In our study the effective filling period was decreased from 34 days at 32/22 °C to 26 days at 40/30 °C. These results suggest that high temperatures reduce the seed size by decreasing the duration of seed-filling, without a compensatory increase in the rate of seed-filling. This is in contrast to wheat which showed that higher temperatures decreased the seed filling duration, while increasing the seed filling rates (Wheeler et al., 1996b).

In conclusion, the seed yield of grain-sorghum will decrease at temperature >32/22 °C at ambient or elevated growth CO_2, due to decrease in pollen viability, seed-set and harvest index. There were negative interactions between elevated temperature and CO_2 on pollen viability, seed-set, harvest index and seed yield and the adverse effects of elevated temperature were more severe at elevated CO_2 than at ambient CO_2. The beneficial effects of elevated CO_2 decreased with increasing temperature. The adverse temperature sensitivity of reproductive processes and yield at elevated CO_2 was due to higher canopy foliage (caused by partial closure of stomata) and seed temperatures.

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